pending. Pursuant to 37 C.F.R. § 1.121 (c), directions to rewrite the claims are given above, and a marked-up version of the amended claims is attached as Appendix A.

Claims 87 and 122 have been amended to remove the subject matter added in the previous Response of February 6, 2001. Claims 13 and 128 have been amended to remove some of the subject matter added in the previous Response of February 6, 2001, particularly the subject matter specifying that the means for optically exciting the sample operates "during at least a portion of the thermal cycling." Claims 13 and 128 have been further amended to specify that the means for detecting the fluorescence operates "during amplification." Additionally, claims 13 and 128, as well as dependent claims 14-32 and 129-144 have been clarified by removing the term "in real time" from the preamble. No new matter is added by way of these amendments.

In addition, claims 19 and 131 have been amended for consistency with previous amendments to their base claims, and claims 22, 24, 82, and 121 have been amended to correct informalities. Finally claim 136 has been amended to change "second" to "first." In the previous Response of February 6, 2001, claims 24 and 136 were amended to specify that the means for optically exciting the sample impinges the first side of the sample container. However, the amendment to claim 136 inadvertently indicated the second side of the sample container. The present amendment to claim 136 is made to correct this prior typographical error. No change in scope is intended by way of these amendments.

Claims 157 and 158 have been added to specify that the means for detecting the fluorescence of the excited sample during amplification detects fluorescence throughout temperature cycling or during an extension or combined annealing/extension phase of temperature cycling, respectively. Support for new claim 157 is found in figures 12 and 13, and in the specification at page 79, line 13 to page 81, line 13, wherein amplification

reactions are monitored continuously throughout temperature cycling, and fluorescence is acquired every 200 msec. Support for new claim 158 is found in the specification at page 62, lines 4-7 ("To carry out such cycle-by-cycle monitoring, fluorescence is acquired during the extension or combined annealing/extension phase of each cycle. . . ."). Examples of once-per-cycle monitoring are plotted in figure 14.

No new matter is added by way of any of the amendments.

Claims 13-32, 87-92, 122-144, and 152-156 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. In particular, the examiner contends that the claim amendment entered in the previous response, requiring that the light source is positioned to excite the sample during at least a portion of the temperature cycling, constitutes new matter. Applicants have amended the claims to delete this language, thus obviating the rejection.

Furthermore, applicants note that the new matter rejection only applies to the amendment to the light source or means for optically exciting element, and does not extend to the detector or means for detecting element. In claims 13-32 and 128-144, Applicants have amended the means for detecting element to clarify that the means for detecting the fluorescence of the excited sample operates during amplification. As cited in the previous response, support is found in the specification on pages 76-77 and figure 11. In particular, the fluorescence is detected via photomultiplier tubes 362A&B. When multiple samples are used, a carousel positions each sample container (capillary sample tube) sequentially at a monitoring location for a 10-100 msec acquisition. For continuous monitoring of a single sample, the sample container is held in a monitoring position with data acquired every 200

msec. Time, temperature, and fluorescence may be continuously displayed as fluorescence vs. cycle number and fluorescence vs. temperature plots. As described throughout the specification, fluorescence vs. cycle number plots are plots of fluorescence detected during amplification. Such detection during amplification may be continuous, as reflected in new claim 157, or may be during an extension or combined annealing/extension phase of temperature cycling, as reflected in new claim 158. Applicants respectfully request withdrawal of the new matter rejection under 35 U.S.C. § 112, first paragraph.

Claims 13-32 and 128-144 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner asserts that the term "real time" is vague or indefinite. Applicants have deleted the term "in real time" from these claims. Thus, applicants respectfully request withdrawal of this rejection.

Claims 13, 18, 20, 28, 128, 129, and 140 stand rejected under 35 U.S.C. § 103(a) as being obvious over either Jerman (U.S. Patent No. 5,824,204) or Schnipelsky (U.S. Patent No. 5,229,297).

First of all, applicants note that claim 28 was amended in the previous response to depend from claim 14, rather than claim 13. Also, claim 128 is directed to the subject matter of claim 15, except written in independent form, and claims 129 and 140 depend therefrom. Claims 14 and 15 clearly contain limitations not disclosed or suggested in either Jerman or Schnipelsky, and, appropriately, have not been included in this rejection. As claims 28, 128, 129, and 140 also include one or more of these limitations, applicants respectfully request withdrawal of this rejection as it pertains to claims 28, 128, 129, and 140.

Next, as amended, each of claims 13, 18, 20, 28, 128, 129, and 140 require means for detecting the fluorescence of the excited sample during amplification. Neither

Jerman nor Schnipelsky teach monitoring the reaction during amplification. Schnipelsky in no way suggests monitoring the reaction during amplification, while Jerman specifically teaches monitoring the post-amplification fluorescent products of the reaction (see col. 5, lines 43-46, "After the desired reaction products are made, they are transported to the injection zone . . . followed by electrophoretic separation and subsequent detection."). Thus, as amended, claims 13, 18, 20, 28, 128, 129, and 140 are not obvious in light of Jerman or Schnipelsky.

Finally, each of claims 13, 18, 20, 28, 128, 129, and 140 require means for positioning the PCR sample container in a monitoring position. Both Jerman and Schnipelsky disclose closed systems for performing PCR wherein the sample itself is moved between various chambers within the closed device. The sample itself is moved within the container, and neither Jerman nor Schipelsky teach means for positioning the container in a monitoring position. Furthermore, Jerman teaches analysis of the products by capillary electrophoresis, which teaches away from moving the entire device to a detector. Schnipelsky teaches a cuvette with a detection compartment. A cuvette is generally handled manually, and Schnipelsky does not disclose any means for moving the cuvette to a monitoring position. Additionally, Schnipelsky does not teach a device formed for holding less than 1 milliliter of sample. Rather Schnipelsky discloses a cuvette with a volume of about 200 ml (col. 8, line 60). Thus, neither Jerman nor Schnipelsky disclose or suggest the invention of claims 13, 18, 20, 28, 128, 129, and 140.

Applicants respectfully request withdrawal of claims 13, 18, 20, 28, 128, 129, and 140 under 35 U.S.C. § 103 (a).

CONCLUSION

The amendments and remarks presented herein are intended to fully address each of the Examiner's objections/rejections. In light of the amendments and remarks, the applicants respectfully request allowance of the pending claims and passage of the application to issuance.

Respectfully submitted,

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INDS02 JTP 386871

APPENDIX A Marked-Up Version of Rewritten Claims

13. (Thrice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling comprising;

a sample container for holding a PCR sample, the sample container comprising an optically clear material, the sample container formed for holding less than 1 milliliter of a sample and having a first side, a second side, and an end;

means for positioning the PCR sample container in a monitoring position; means for heating the PCR sample;

means for cooling the PCR sample;

control means for repeatedly operating the means for heating and the means for cooling to subject the PCR sample to thermal cycling;

means for optically exciting the sample [during at least a portion of the thermal cycling] to cause the sample to fluoresce; and

means for detecting the fluorescence of the excited sample during

amplification [at least a portion of the thermal cycling] when the sample is in the monitoring position.

- 14. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 further comprising: means for determining at least one reaction parameter in accordance with the detected fluorescence.
- 15. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 14 further comprising means for adjusting the control means in accordance with the reaction parameter.

16. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 15 in which the control means adjusts the operation of the means for heating and the means for cooling to alter the times the means for heating and the means for cooling operate in accordance with the reaction parameter.

- 17. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 15 in which the control means adjusts the operation of the means for heating and the means for cooling to alter the rate at which the biological sample is heated and cooled in accordance with the reaction parameter.
- 18. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the sample container is fabricated at least partially from glass, the sample container having a volume not greater than about 10,000.
- 19. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the means for positioning the PCR sample container in a monitoring position comprises a rotatable carousel.
- 20. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 further comprising means for positioning the means for optically exciting the sample and the means for detecting the fluorescence of excited sample to optimize the fluorescence which is detected.
- 21. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the means for heating the PCR sample comprises a forced air heater.

22. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as [denied] <u>defined</u> in claim 13 wherein the means for cooling comprises an air movement mechanism which transports ambient air to the sample container.

- 23. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the control means comprises a microprocessor.
- 24. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as [denied] <u>defined</u> in claim 13 wherein the means for optically exciting the sample comprises a photo emitter structure positioned so that the radiation emitted therefrom impinges the first side of the sample container.
- 25. (Thrice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 24 wherein means for detecting the fluorescence of the excited sample comprises a photo detector structure positioned so that the radiation emitted from the second side of the sample container is detected.
- 26. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the means for optically exciting the sample comprises a photo emitter structure positioned so that the radiation emitted therefrom impinges the end of the sample container.
- 27. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 26 wherein the means for detecting the fluorescence of the excited sample comprises a photo detector structure positioned so that the radiation emitted from the end of the sample container is detected.

28. (Thrice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 14 wherein the means for determining at least one reaction parameter in accordance with the detected fluorescence comprises means for determining at least one reaction parameter selected from the group consisting of: product melting temperature, product melting time, product reannealing temperature, product reannealing time, primer annealing/extension temperature, and primer annealing/extension time.

- 29. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the control means comprises means cooling the sample when the means for detecting the fluorescence of the excited sample detects that the product is completely melted.
- 30. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the control means comprises means for heating the sample when the means for detecting the fluorescence of the excited sample detects no more product generation.
- 31. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the means for optically exciting is positioned to interact with the first side of the sample container and the means for detecting the fluorescence is positioned to interact with the second side of the sample container.
- 32. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 13 wherein the means for optically exciting is positioned to interact with the end of the sample container and the means for detecting the fluorescence is positioned to interact with the end of the sample container.

82. (Twice Amended) A device for conducting PCR reactions, said device comprising a chamber;

a heater and a fan mounted in said device and in air flow communication with the chamber;

<u>a</u> carousel for holding a plurality of sample vessels, said carousel being rotatably mounted in said chamber;

each of said sample vessels comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of each of said sample vessels is less than 1mm;

a light emitting source mounted in said chamber and positioned to illuminate at least one selected sample vessel along an axis substantially parallel to a wall along the second dimension of the selected sample vessel; and

a light detector mounted in said chamber and positioned to measure fluorescence from the selected sample vessel along an axis substantially parallel to a wall along the second dimension of the selected sample vessel.

87. (Thrice Amended) A system for performing PCR and monitoring the reaction comprising;

a chamber;

a heater and a fan in air flow communication with the chamber and a controller for cycling the temperature in the chamber according to initial predefined temperature and time parameters;

a carousel for holding a plurality of sample vessels said carousel being rotatably mounted in said chamber, said sample vessels comprising an optically transparent

material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1mm;

a light emitting source mounted in said chamber and positioned to illuminate at least one of the sample vessels [during at least a portion of the temperature cycling] along an axis substantially parallel to a wall along the second dimension of the vessel;

a light detector mounted in said chamber and positioned to measure fluorescence from at least one of the sample vessels [during at least a portion of the temperature cycling] along an axis substantially parallel to a wall along the second dimension of the vessel; and

means for displaying the status of the reaction based on detected fluorescence.

121. (Amended) A device for conducting PCR reactions, said device comprising

a chamber;

a heater and a fan mounted in said device and in air flow communication with the chamber;

<u>a</u> carousel for holding a plurality of sample vessels, said carousel being rotatably mounted in said chamber;

said sample vessels comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of each of said sample vessels is less than 1mm;

a light emitting source positioned to illuminate at least one selected sample vessel along an axis substantially parallel to a wall along the second dimension of the selected sample vessel; and

a light detector positioned to measure fluorescence from the selected sample vessel along an axis substantially parallel to a wall along the second dimension of the selected sample vessel.

122. (Twice Amended) A system for performing PCR and monitoring the reaction comprising:

a chamber;

a heater and a fan in air flow communication with the chamber and a controller for cycling the temperature in the chamber according to initial predefined temperature and time parameters;

a carousel for holding a plurality of sample vessels said carousel being rotatably mounted in said chamber, said sample vessels comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1mm;

a light emitting source positioned to illuminate at least one of the sample vessels [in the chamber during at least a portion of the temperature cycling] along an axis substantially parallel to a wall along the second dimension of the vessel;

a light detector positioned to measure fluorescence from at least one of the sample vessels [in the chamber during at least a portion of the temperature cycling] along an axis substantially parallel to a wall along the second dimension of the vessel; and means for displaying the status of the reaction based detected fluorescence.

128. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling comprising;

a sample container for holding a PCR sample, the sample container comprising an optically clear material, the sample container formed for holding less than 1 milliliter of a sample and having a first side, a second side, and an end;

means for positioning the PCR sample container in a monitoring position; means for heating the PCR sample;

means for cooling the PCR sample;

control means for repeatedly operating the means for heating and the means for cooling to subject the PCR sample to thermal cycling;

means for optically exciting the sample [during at least a portion of the thermal cycling] to cause the sample to fluoresce;

means for detecting the fluorescence of the excited sample during

amplification [at least a portion of the thermal cycling] when the sample container is in the monitoring position;

means for determining at least one reaction parameter in accordance with the detected fluorescence; and

means for adjusting the control means in accordance with the reaction parameter.

129. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 in which the control means adjusts the operation of the means for heating and the means for cooling to alter the times the means for heating and the means for cooling operate in accordance with the reaction parameter.

130. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 in which the control means adjusts the operation of the means for heating and the means for cooling to alter the rate at which the biological sample is heated and cooled in accordance with the reaction parameter.

- 131. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the means for positioning the PCR sample container in a monitoring position comprises a rotatable carousel.
- 132. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 further comprising means for positioning the means for optically exciting the sample and the means for detecting the fluorescence of excited sample to optimize the fluorescence which is detected.
- 133. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the means for heating the PCR sample comprises a forced air heater.
- 134. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the means for cooling comprises an air movement mechanism which transports ambient air to the sample container.
- 135. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the control means comprises a microprocessor.
- 136. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the means

for optically exciting the sample comprises a photo emitter structure positioned so that the radiation emitted therefrom impinges the [second] <u>first</u> side of the sample container.

- 137. (Twice Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 136 wherein means for detecting the fluorescence of the excited sample comprises a photo detector structure positioned so that the radiation emitted from the second side of the sample container is detected.
- 138. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the means for optically exciting the sample comprises a photo emitter structure positioned so that the radiation emitted therefrom impinges the end of the sample container.
- 139. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 138 wherein the means for detecting the fluorescence of the excited sample comprises a photo detector structure positioned so that the radiation emitted from the end of the sample container is detected.
- [in real time] during temperature cycling as defined in claim 128 wherein the means for determining at least one reaction parameter in accordance with the detected fluorescence comprises means for determining at least one reaction parameter selected from the group consisting of: product melting temperature, product melting time, product reannealing temperature, product reannealing time, primer annealing/extension temperature, and primer annealing/extension time.
- 141. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the control means

comprises means cooling the sample when the means for detecting the fluorescence of the excited sample detects that the product is completely melted.

- 142. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the control means comprises means for heating the sample when the means for detecting the fluorescence of the excited sample detects no more product generation.
- [in real time] during temperature cycling as defined in claim 128 wherein the means for optically exciting is positioned to interact with the first side of the sample container and the means for detecting the fluorescence is positioned to interact with the second side of the sample container.
- 144. (Amended) A system for performing PCR and monitoring the reaction [in real time] during temperature cycling as defined in claim 128 wherein the means for optically exciting is positioned to interact with the end of the sample container and the means for detecting the fluorescence is also positioned to interact with the end of the sample container.
- 152. (Twice Amended) A system for performing PCR and monitoring the reaction comprising:

a chamber;

a heater and a fan in air flow communication with the chamber and a controller for cycling the temperature in the chamber according to initial predefined temperature and time parameters;

a carousel for holding a plurality of sample vessels said carousel being rotatably mounted in said chamber; the carousel comprising a disc having a top surface, a

bottom surface, and an outer edge extending therebetween, a sample receiving port in the top surface, a sample vessel port in the outer edge, and a sample passageway communicating with said sample receiving port and the sample vessel port, said sample vessel port and passageway formed for receiving and fixing a sample vessel to the disc; the passageway including a barrier that prevents a liquid sample delivered through the sample receiving port from flowing to the sample vessel port absent a biasing force on said liquid sample;

said sample vessels comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1mm;

a light emitting source positioned to illuminate at least one of the sample vessels [in the chamber during at least a portion of the temperature cycling] along an axis substantially parallel to a wall along the second dimension of the vessel;

a light detector positioned to measure fluorescence from at least one of the sample vessels [in the chamber during at least a portion of the temperature cycling] along an axis substantially parallel to a wall along the second dimension of the vessel; and

a display for displaying the status of the reaction based detected fluorescence.